Solvents Based Extraction of Antioxidants and their Activity from Some Plants of Cholistan Desert, Pakistan

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ABSTRACT

A project was designed to investigate the effect of extraction solvents on phytochemical extraction of plants endemic to the desert habitat of Cholistan Desert, Pakistan. In this study four types of solvents Chloroform, Acetone, Propanol, and Acetic acid were used to examine total antioxidant activity contents, total flavonoid contents, and total tannin contents. Plants typically of the desert area were selected. Keeping in view the similar age of plant and their parts the specimens were collected with three replicates of each. Specimens after proper identification and labeling were processed for extraction. Results showed that extraction solvents had significant differences for extraction of total antioxidant activity contents, total flavonoid contents, and total tannin contents. Acetone showed maximum total antioxidant activity contents and Chloroform showed maximum total flavonoid contents. Minimum total antioxidant activity contents and total flavonoid contents were measured by extraction in Acetic acid. Maximum total tannin contents were measured by solvent Propanol.

Key Words: Solvent, extraction, phytochemicals, Cholistan, desert.

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INTRODUCTION

The plants growing in deserted soils are rich in phenolic acids, flavonoids, and tannins [1, 2] because desert plants have to face all types of abiotic stresses like high temperature, light, salts concentration, and water shortage. These stresses produce reactive oxygen species which have damaging effects to plants in response to which plant produce defensive chemicals like antioxidants phenolic acids and flavonoids, tannins for their defense [1]. In plants, naturally occurring antioxidants are of two types 1) enzymatic antioxidants include superoxide dismutases, catalases, peroxidases, etc 2) Non-enzymatic antioxidants include Alkaloids, phenols, flavonoids, tannic acids, etc. Both of these play an important role as natural antioxidants [3]. This also disturbs the survival of different plants and crops [4]. Ingestion of bioactive compounds containing food has the potential of antioxidants and can control human diseases such as heart diseases and cancer [5, 6]. Plants have been reported as a source of product effective for the treatment of many other diseases [7, 8]. Plant-derived medicines have an advantage over synthetic medicines due to having a low toxicity [9]. Also, the diversity of plant secondary metabolites that result from plant evolution is superior to that found in synthetic drugs. Despite a wide historical background, there are only a few plants that have been studied for their drug potential. The medicinal potential of the plants can be assessed by the chemical agents they possess which may alter their certain physiologic effects in the body of humans. Some of the most important of these constituents of plants are terpenes, alkaloids, flavonoids, and phenolic compounds. A variety

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of plants contain different phytochemicals that can be of therapeutic value [10].

The plants are a potential source of many drugs and in recent years there has been an increasing trend about the use and importance of medicinal plants. Medicines obtained from the plants are less expensive, safer, and rarely have side effects. The plants have been selected for medicinal use over thousands of years for various drugs such as anticancer drugs [11] and antimicrobial drugs [12]. However, medicinal plants should be investigated to better understand their properties, safety, and efficiency [13].

MATERIALS AND METHODS

Plant sample collections

After a preliminary survey of Cholistan desert, fresh shoot specimens were collected and identification of the plants was performed by matching them with the labeled herbarium exsiccates lying in the departmental herbarium (Dr.Mumtaz Bukhari herbarium) of Botany Department Bahauddine Zakarya University, Multan Pakistan and/or the literature [14]. Data and specimens were collected according to an appropriate methodology [15, 16] keeping uniformity among the age of plants, size of plants, and size of dunes. Further processing of collected specimens was carried out in the laboratory of the department. The specimens were first washed with water and later with 2% Ethanol to remove dust and other surface contaminants, dried at room temperature and were pulverized to a fine powder by using pestle and mortar.

Extraction

For the extraction, 50.0 gm plant powder was taken in flask then 300 ml of each solvent was poured. All the flasks were cotton plugged to prevent solvent from evaporation. The flasks were placed on an orbital shaker. Then left the shaker for the whole night to obtain a good quality extract. After that the flasks were removed from the shaker and the extract transferred to clean bottles carefully. The extract was stored on - 4 ^{o}C.

Determination of total antioxidant activity

By using the method of Govindarajan [17] and Subhasree *et al.,* [18]. The extracted material was used to a solvent dilution after that 0.2 ml was taken from that diluted extract. In a plastic tube 1.8 ml of Phosphomolybdate reagent (0.6 M Sulfuric acid, 4.0 mM Ammonium molybdate and 28 mM Sodium phosphate) and 0.2 ml diluted extract mixed well at 90 ^{o}C for 90 minutes in a water bath. Total antioxidant activity was measured by using a Hitachi U-2900 spectrometer, the absorbance was taken at 695 nm. The calibration curve was prepared using Ascorbic acid (5-60 mg/ml) as the standard.

Determination of total flavonoids contents

By using Aluminum Chloride Method [19], Total flavonoid content was estimated. The sample was diluted with a solvent. 0.5 ml extract, 0.1 ml of 10% (w/v) Aluminum chloride solution, 0.1 ml of 0.1 mM Potassium acetate solution and extract was then mixed. The whole solution was placed at room temperature. The mixture solution was kept at room temperature for 25 minutes. Then by using a Hitachi U-2900 spectrophotometer, the absorbance of the sample was recorded at 415 nm. The calibration curve was made using Quercetin (0-100 mg/ml).

Determination of total tannin contents

Tannins can be documented in plants by Swain's method [20]. In a flask, 1.0 ml of sample extract and added 20 ml water to dilute it and followed it by 2.5 ml of Folin Denis reagent and 10 ml Na_2CO_3 mixed well and allowed to stand it at room temperature. After 20 minutes of bluish-green color appeared which indicates the presence of tannins. Standard solutions of tannic acid ranging from 0-10 ppm were used for the calibration curve. The absorbance was read at 760 nm by using Hitachi U-2900 spectrophotometer after color development.

Statistical analysis of data

One way ANOVA and least significant differences (LSD) were calculated by using COSTAT at p= 0.05 for all tests [21]. Data were interpreted as mean values and standard deviation (± SD).

RESULTS

Total antioxidant activity contents (mg/10g)

The solvent, Acetone showed maximum (1472.752 mg/10g) while Acetic acid showed minimum (150.323 mg/10g) results for total antioxidant activity contents. Propanol showed maximum (729.030 mg/10g) and Chloroform showed minimum (464.102 mg/10g) mean values but their differences are significant. All four solvents have significant effects on plants in total antioxidant activity contents. Every solvent has shown different behavior for different plants (Table 1b). In a solvent Acetone maximum (2148.502 mg/10g) yield was given in *Calligonum polygonoides* but minimum (613.3 mg/10g) yield was given in *Calotropis procera.* In a solvent Chloroform maximum (931.352 mg/10g) yield was found in *Calligonum polygonoides* while minimum (0.188 mg/10g) yield was found in *Haloxylon stocksii* and they have a highly significant difference.

In a solvent Propanol, maximum (888.376 mg/10g) total antioxidant activity contents were measured in *Calotropis procera* but minimum (621.308 mg/10g) total antioxidant activity contents were measured in *Haloxylon stocksii.* In Acetic acid the maximum (179.487 mg/10g) values were

given in *Salsola imbricata* while minimum (12.9051 mg/10g) values were given in *Calotropis procera*.

The total antioxidant activity contents in plants, maximum (959.776 mg/10g) mean value was found in *Calligonum polygonoides* and minimum (522.982 mg/10g) mean value was found in *Calotropis procera*. *Leptadenia pyrotechnica* showed mean value as (671.452 mg/10g), *Haloxylon stocksii* showed minimum (535.482 mg/10g) mean value while *Salsola imbricata* showed maximum (830.191 mg/10g) mean values. Both plants have strongly different behaviors. *Haloxylon stocksii* showed a percentage difference of 591.70% and *Calotropis procera* showed a significant percentage difference of 593.56% for total antioxidant activity contents between them (Table 1).

Total flavonoid contents (mg/10g)

Maximum and minimum mean values for the effects of solvents on flavonoid contents showed by Chloroform (882.887 mg/10g) and Acetic acid (122.108 mg/10g). The maximum (304.133 mg/10g) value was found by solvent Acetone but minimum (244.915 mg/10g) value was found by solvent Propanol for flavonoid extraction and statistically, all solvents showed significant difference. By using the value of solvent Chloroform as standard value the percentage difference was taken. The solvents Acetone and Propanol have a 6.71% difference between them. The percentage difference is 13.91% between Propanol and Acetic acid.

In a solvent Chloroform, the maximum (2096.922 mg/10g) value for total flavonoid contents was measured in *Leptadenia pyrotechnica* and the minimum (389.731 mg/10g) values were measured in *Calotropis procera.* Both *Salsola imbricata* and *Haloxylon stocksii* showed the same value (632.611 mg/10g) for total flavonoid contents. They both shrubs showed highly non-significant behaviors. *Calligonum polygonoides* showed the value (662.559 mg/10g). In Acetone minimum (144.363 mg/10g) quantity of total flavonoid contents was found in *Calotropis procera* but maximum (441.130 mg/10g) quantity of total flavonoid contents was found in *Haloxylon stocksii.* In a solvent Propanol the difference of plants in terms of total flavonoid contents was maximum (350.926 mg/10g) in *Calligonum polygonoides* but minimum (111.338 mg/10g) were measured in *Leptadenia pyrotechnica.* In Acetic acid the total flavonoid contents were found maximum (153.33 mg/10g) in *Calotropis procera* but the total flavonoid contents were found minimum (77.703 mg/10g) in *Calligonum polygonoides.*

The plants *Leptadaenia pyrotechnica* showed maximum (644.366 mg/10g) mean value while *Calotropis procera* showed minimum (239.674 mg/10g) mean value for total flavonoid contents. The total flavonoid contents mean value were measured maximum (346.615 mg/10g) in *Calligonum polygonoides* and minimum (367.851 mg/10g) in *Salsola imbricata* (Table 2).

Total tannins contents (mg/10g)

Extraction solvent Acetic acid showed maximum (125.701 mg/10g) and Propanol showed minimum (55.474 mg/10g) mean values. Acetone showed maximum (111.491 mg/10g) while Chloroform showed minimum (85.837 mg/10g) mean values. Total tannins contents were measured maximum (91.741 mg/10g) in *Calligonum polygonoides* and minimum (90.054 mg/10g) tannins were measured by *Salsola imbricata. Calotropis procera* showed (89.063 mg/10g).

The maximum (116.925 mg/10g) value was shown in *Leptadenia pyrotechnica* and a minimum (85.346 mg/10g) value was shown in *Haloxylon stocksii.* Maximum (91.741 mg/10g) quantity of total tannins contents was found in *Calligonum polygonoides* and a minimum (90.054 mg/10g) quantity was found in *Salsola imbricata*. *Calotropis procera* showed the mean value of (89.063 mg/10g).

In a solvent Chloroform maximum (90.482 mg/10g) total tannins contents were measured in *Calligonum polygonoides* and minimum (82.284 mg/10g) were measured in *Haloxylon stocksii.* In a solvent Acetone the maximum (127.888 mg/10g) value was found in *Leptadenia pyrotechnica* and a minimum (107.733 mg/10g) was found in *Salsola imbricata.* In a solvent Propanol minimum (49.952 mg/10g) quantity of total tannins contents were measured in *Leptadenia pyrotechnica* and maximum (76.853 mg/10g) quantity was measured in *Haloxylon stocksii.* In a solvent Acetic acid the maximum (172.808 mg/10g) value was found in *Leptadenia pyrotechnica* and minimum (101.072 mg/10g) were found in *Haloxylon stocksii* (Table 3).

DISCUSSION

The desert environments or territories have several abiotic restrictions like salinity, lots of harmful radiations, high or low temperatures, and other different severe and tough conditions. Environmental conditions are theoretically harmful to plants proved by Seigler's experiment [22]. These conditions also increase the production of phenylpropanoids [23]. The present study is conducted to study the effects of different solvents on total antioxidants, flavonoids, and tannins. In this experiment plants divided into shrubs, trees, and herbs from the Cholistan desert.

Several studies have shown that phenolic compounds varied with solvents polarities. For example, 50% acetone for extraction of total phenolics [24] and pure methanol was used for the extraction of polyphenols [25] which were found to be more effective than water. Flavonoids act as protective agents against water deficit stress. The flavonoids also protect plants from toxic effects of heavy metals [1]. To extract these bioactive secondary metabolites different solvents have been used. Solvents used for extraction are Chloroform, Ethanol, Methanol,

and Acetone. Terpenoids, Flavonoids can be extracted by Chloroform, Tannins, Polyphenols, Alkaloids, Polyacetylenes, Terpenoids, Sterols can be extracted by Ethanol, Anthocyanins, Terpenoids, Saponins, Tannins, Xanthoxyllines can be extracted by Methanol and Phenol, and Flavanols can be extracted by Acetone. Maximum organic molecules are moderately non-polar and are ordinarily soluble in organic solvents (*e.g.* dichloromethane, chloroform, petroleum ether, hexanes, diethyl ether, etc.) but not in water which is polar solvents. For extraction the solvent type used is significant for both classification and quantification of phenolic compounds present in plants, attaining uncontaminated compounds for their analysis [26]. Several solvents in general used for extraction of different phenolic compounds including water [27] acetone or ethanol-water water [28, 29].

Medicinal plants contain some organic compounds such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids which provide definite physiological action on the human body [30, 31]. These are synthesized by primary or secondary metabolism of plants. Secondary metabolites are chemically and taxonomically extremely diverse compounds that are widely used in human therapy, veterinary, agriculture, scientific research, and many other purposes [32]. These phytochemicals have been shown to have inhibitory effects on microorganisms in vitro [33]. Nutritional antioxidants, as well as polyphenolic compounds, vitamins E and C, and carotenoids, are thought also to be the active and effective nutrients in the avoidance of oxidative stress-related diseases [34, 35]. The phenolic content of the plant determines its antioxidant activity [36]. The relationship between the number of phenolic compounds and antioxidant activity are correlated according to some authors while the others found no correlation or only a weak relation one as some other substances including tocopherols and β-carotene are involved in raising the antioxidant status [37].

Therefore, The knowledge of the phytochemicals is desirable, not only for their importance as therapeutic agents but also because such information may be of value in disclosing new sources of economic materials, such as oils, tannins, gums and as precursors for the synthesis of many complex chemical substances.

REFERENCES

[1] Shirley WB. Flavonoid biosynthesis, a colorful model for genetics, biochemistry, cell biology and biotechnology, 2001, 485-93.

[2] Nassar MI, Ghazawy NA, Torkey HM, Rawy SM. Assessment of Biorational Botanical Extracts on the Desert Locust Schistocerca Gregaria Forskal (Orthoptera: Acrididae. Entomol. appl. sci. lett. 2018;5(2):42-54.

[3] Ito N, Fukushima S, Tsuda H. Carcinogenicity and modification of the carcinogenic response by BHA and BHT and other anti-oxidants, CRC Critical Review of Toxicology, 1985, 15: 109−150.

[4] Gosal S, Wani SH et al. Water and agricultural sustainability strategies" Kang MS, Ed. CRC Press, 2010, 259. 10.4161/psb.5.6.11398.

[5] Temple NJ. Antioxidants and disease: more questions than answers, Nutrition Research, 2000, 20(3): 449-459.

[6] Bhat SH, Ullah MF, Abu-Duhier FM. Anti-hemolytic Activity and Antioxidant Studies of Caralluma quadrangula: Potential for Nutraceutical Development in Cancers and Blood Disorders. Int. J. Pharm. Res. Allied Sci. 2019;8(4):121-9.

[7] Newman, DJ., Cragg, GM. In Drug Discovery, Therapeutics, and Preventive Medicine, L. Zhang, A. Fleming, A. L. Demain (Eds.), Humana Press, Totowa, NJ. In press. 2005.

[8] Nailya S, Zhanat S, Raushan T, Asker T. Polyphenolic Compouds of Plant of Lepidium Ruderale Linn. and Their Biololical Activity. J. Biochem. Technol. 2018;9(4):77-80.

[9] Alduhisa GU, Demayo CG. Ethnomedicinal Plants Used By The Subanen Tribe In Two Villages In Ozamis City, Mindanao, Philippines. Pharmacophores. 2019;10(4):28-42.

[10] Gurib-Fakim A. Medicinal plants: Tradition of yesterday and drugs of tomorrow, Molecular Aspects of Medicin, 2006, 27 (1):1-93.

[11] Dewick PM. Tumor inhibition from plants: 2006, Tease and Evans.

[12] Phillipson JD., Wright CW. Plants With Antiprotozoal Activity, Tease and Evans, Pharmacognosy, 14th edn., WB Saunders Company, London, 1996, pp: 612.

[13] Arunkumar S., Muthuselvam Analysis of phytochemical constituents and antimicrobial activities of aloe vera L. against clinical pathogens, World Journal of Agricultural Science, 2009, 5(5): 572-576.

[14] Ali SI., Qaiser M. Flora of Pakistan. Department of Botany, University of Karachi, Pakistan. Nos. 1993, 2011: 194–218.

[15] Jain SK. Ethnobotany: Its Scope and Study. Indian Museum Bulletin, 1967, 2: 39-43.

[16] Khan SS. Ethnomedicinal studies on plants of Bhopal district of M.P. Ph.D.Thesis, Barkatullah University, Bhopal.1993.

[17] Govindarajan R, Rastogi S, Vijayakumar M, Shirwaikar A, Rawat AK, Mehrotra S, Pushpangadan P. Studies on the antioxidant activities of Desmodium gangeticum Biological and Pharmaceutical Bulletin,2003, 26(10): 1424-1427.

[18] Subhasree B, Baskar R, Laxmi Keerthana R. Evaluation of antioxidant potential in selected green leafy vegetables. Food Chemistry, 2009, 115(4): 1213-1220.

[19] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis,2002, 10(3): 178-82.

[20] Swain T., Hills E. The phenolic constituents of Prunus domestica. The quantitative analysis of phenolic constituents, Journal of the Science of Food and Agriculture, 1959, 10: 63-68.

[21] Snedecor GW., Cochran WG. In: Statistical Method. 7th Edition. The Iowa State University Press, Ames. 1980.

[22] Seigler DS. Plant Secondary Metabolism, Boston MA: Chapman and Hall (Kluwer Academic Publishers). 1998.

[23] Dixon RA., Paiva N. Stressed induced phenylpropanoid metabolism, Plant Cell,1995, 7: 1085-97.

[24] Zhou K., Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation- LWT, Food Science and Technology, 2004, 37(7): 717–721.

[25] Yao LH, Jiang YM, Caffin N, D'arcy B, Datta N, Liu X, Singanusong R, Xu Y. Phenolic compounds in tea from Australian supermarkets, Food Chemistry 2006, 96(4): 614–620.

[26] Brun N, Jay M, Merghem R. A proposition for the study of phenolic and of legumes, In Proceeding of Ist European Conference on Grain Legumes, Anger, France, 1992, pp: 393-394.

[27] Matsuzaki T., Hara Y. Antioxidant activity of tea leaf catechins, Nippon Nogeikagu Kaishi., 1985,59: 129-134.

[28] Saijo R. Isolation and chemical structures of two new catechins from fresh tea leaf, Agricultural and Biological Chemistry,1982, 46(7): 1969-1970.

[29] Amarowicz R, Piskula M, Honke J, Rudnicka B, Troszynska A, Kozlowska H. Extraction of phenolic compounds from lentil seeds (Lens culinaris) with various solvents, Polish Journal of Food & Nutrition Science, 1995, 4(45): 53-62.

[30] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemicals constituents of some Nigerian medicinal plants, African Journal of Biotechnology, 2005, 4(7): 685-688.

[31] Mann J. Secondary Metabolism, Oxford University Press, London, 1978, pp. 154.

[32] Goud JV, Suryam A, Charya MS. Biomolecular and phytochemical analyses of three aquatic angiosperms, African Journal of Microbiology Research, 2009, 3(8):418-421.

[33] Cowan MM. Plant products as antimicrobial agents, Clinical Microbiology Review. 1999, 12(4): 564-582.

[34] Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proceedings of National Academy of Science. U.S.A.,1995, pp: 5258-5265.

[35] Kaur C, Kapoor HC. Antioxidants in fruits and vegetables the millennium's health. International Journal of Food Science and Technology, 2001, 36(7): 703-725.

[36] EREL ŞB, Reznicek G, Şenol SG, YAVAŞOĞLU NÜ, Konyalioğlu S, Zeybek AU. Antimicrobial and antioxidant properties of Artemisia L. species from western Anatolia. Turkish Journal of Biology, 2012, 36(1): 75-84.

[37] Gursoy N, Sarikurkcu C, Cengiz M, Solak MH. Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. Food and Chemical Toxicology, 2009, 47(9): 2381-2388.

Table 1: Effect of extraction solvents on total antioxidant activity contents (mg/10g) of plants Cholistan Desert, Pakistan.

	Chloroform	Acetone	Propanol	Acetic acid	(MEANS) (LSD= 36.805)
Leptadenia pyrotechnica	488.937 ± 60.748 (h)	1220.53 ± 60.962 (d) (239.30)	812.284 ± 51.877 (f) (322.80)	164.059 ± 4.176 (i) (455.382)	671.452 ± 410.816 (c)
Calligonum polygonoides	931.352 ± 27.092 (e)	2148.502 ± 42.125 (a) (700.66)	624.292 ± 70.818 (g) (864.32)	134.959 ± 3.234 (i) (916.86)	959.776± 776.666 (a) (528.51)
Haloxylon stocksii	0.188 ± 0.010 (i)	1376.23 ± 51.381 (c) (-732037.04)	621.308 ± 65.017 (g) (-330482.79)	144.203 ± 4.952 (i) (76703.53)	535.482 ± 562.087 (d) (591.70)
Salsola imbricata	438.685 ± 33.224 (h)	2003.701 ± 56.116 (b) (-18.06)	698.892 ± 76.135 (g) 0.14	179.487 ± 1.845 (i) (397.77)	830.191 ± 734. 730 (b) (547.81)
Calotropis procera	461.348 ± 50.512 (h)	613.3 ± 58.403 (g) (328.41)	888.376 ± 95.720 (ef) (268.78)	12.9051 ± 5.190 (i) (458.55)	522.982 ± 291.174 (d) (593.56)
(MEANS) (LSD= 41.150)	464.102 ± 307.128 (c)	1472.752 ± 578.161 (a) (141.703)	729.030 ± 125.698 (b) (307.01)	150.323 ± 19.784 (d) (431.71)	703.977 ± 590.258 (312.4)

Means followed by dissimilar letters, are different at p= 0.05 (LSD).
Values represent mean ± SE; values in parentheses represent %age increase (+)/ decrease (-) over column 1(chloroform).

Table 2: Effect of extraction solvents on total flavonoid contents (mg/10g) of selected plants from Cholistan Desert, Pakistan.

	Chloroform	Acetone	Propanol	Acetic acid	(MEANS) (LSD= 416.904)
Leptadenia pyrotechnica	2096.922 ± 2516.120 (a)	219.393 ± 2.761 (b) (2086.45)	111.338 ± 1.794 (b) (2091.61)	149.81 ± 4.009 (b) (2089.77)	644.366 ± 1385.622 (a)
Calligonum polygonoides	662.559 ± 10.657 (b)	295.276 ± 1.777 (b) (617.99)	350.926 ± 1.740 (b) (609.59)	77.703 ± 3.264 (b) (650.83)	346.615 ± 218.388 (a) (590.57)
Haloxylon stocksii	632.611 ± 26.642 (b)	441.130 ± 2.171 (b)	223.783 ± 2.224 (b)	78.663 ± 2.331 (b)	344.047 ± 220.373 (a) (590.97)
Salsola imbricate	632.611 ± 18.786 (b)	420.501 ± 2.022 (b)	267.257 ± 1.191 (b)	151.036 ± 2.480 (b)	367.851 ± 188.467 (a) (587.27)
Calotropis procera	389.731 ± 224.700 (b)	144.363 ± 3.772 (b)	271.271 ± 1.449 (b)	153.33 ± 1.92 (b)	239.674 ± 141.801 (a) (607.17)
(MEANS) (LSD= 466.113)	882.887 ± 1147.599 (a)	304.133 ± 118.126 (b)	244.915 ± 81.149 (b)	122.108 ± 37.226 (b)	388.511 ± 636.349 (584.07)

Means followed by dissimilar letters, are different at p= 0.05 (LSD).
Values represent means ± SE; values in parentheses represent %age increase (+)/ decrease (-) over column 1(chloroform)

Table 3: Effect of extraction solvents on total tannin contents (mg/10g) of selected plants from Cholistan Desert, Pakistan.

	Chloroform	Acetone	Propanol	Acetic acid	(MEANS) LSD= 13.043
Leptadenia pyrotechnica	90.141 ± 5.938 (cde)	127.888 ± 5.938 (b) (-51.734)	76.853 ± 45.815 (ef) (4.88)	172.808 ± 55.792 (a) (-101.56)	116.925 ± 49.782 (a)

Calligonum polygonoides	90.482 ± 7.708 (cde)	105.000 ± 5.827 (bcde) (-125.56)	50.084 ± 0.119 (f) (35.129)	121.397 ± 25.580 (b) (-43.68)	91.741 ± 29.957 (b) (38.46)
Haloxylon stocksii	82.284 ± 4.357 (de)	108.075 ± 4.870 (bcd) (-49.05)	49.952 ± 0.092 (f) (21.57)	101.072 ± 4.611 (bcde) (-40.54)	85.346 ± 23.752 (b) (43.93)
Salsola imbricate	83.48 ± 5.667 (e)	107.733 ± 3.074 (bcd) (-45.57)	49.997 ± 0.095 (f) (23.58)	119.006 ± 6.148 (bc) (-59.09)	90.054 ± 27.886 (b) (39.90)
Calotropis procera	82.796 ± 3.845 (de)	108.758 ± 6.148 (bcd) (-48.56)	50.476 ±0.051 (f) (21.83)	114.224 ± 4.620 (bc) (-55.16)	89.063 ± 26.620 (b) (40.75)
(MEANS) LSD= 14.583	85.837 ± 6.116 (c)	111.491 ± 9.684 (b) (-44.04)	55.474 ± 20.553 (d) (21.20)	125.701 ± 34.597 (a) (-60.60)	94.626 ± 33.801 (35.99)

Means followed by dissimilar letters, are different at p= 0.05 (LSD).
Values represent means ± SE; values in parentheses represent %age increase (+)/ decrease (-) over column 1(chloroform).